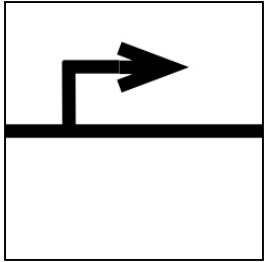
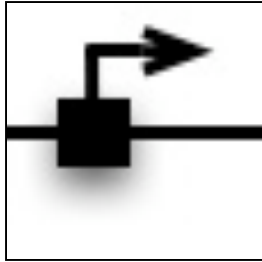


Construction Plan Overview

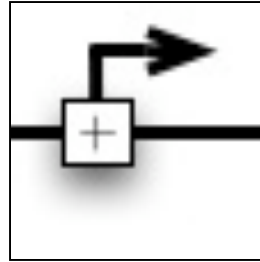
Quick vBOL Key



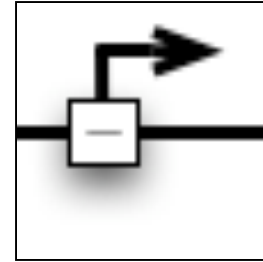
Forward Promoters



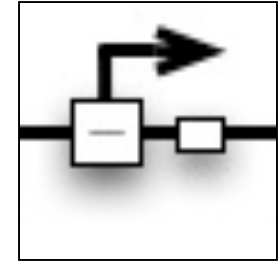
Constitutive



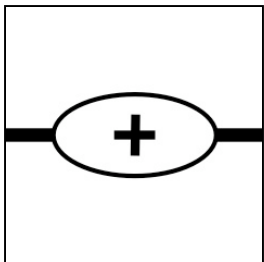
Inducible



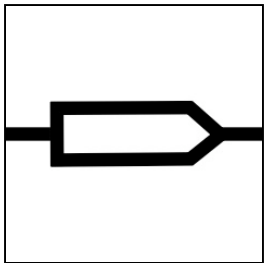
Repressible



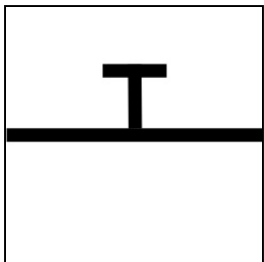
Promoter +
Operator



Forward Ribosome Binding Sight



Open Reading Frame (gene)



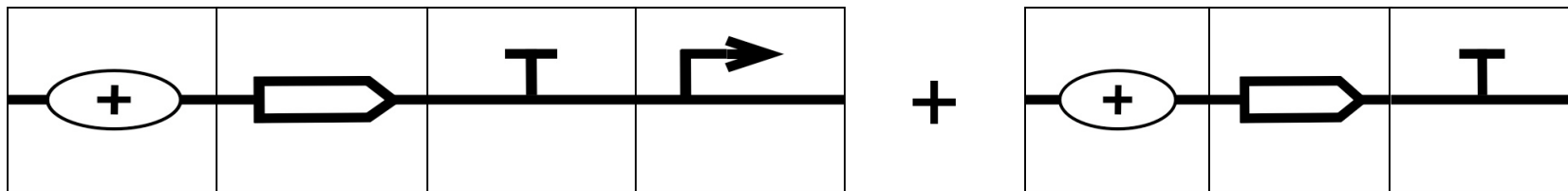
Terminator

Device 1

First Round of Cloning

1.1a SoxR/S Promoter + GFP tag

(Synthesized part # 54) + (BioBrick part # I13504)



B0034 + k223000 + B0015 + k2230001

(RBS) (SoxR gene) (TT) (SoxS Promoter)

Cut w/ EcoR1/Spe1

B0034 + E0040 + B0015

(RBS) (GFP) (TT)

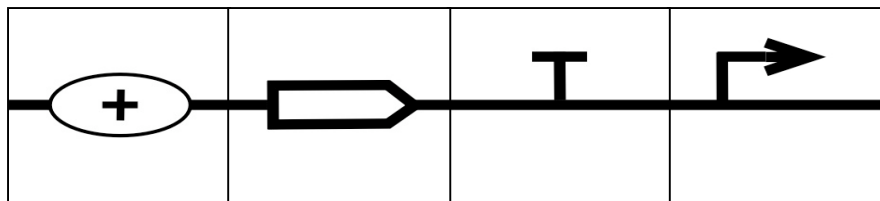
Cut w/ EcoR1/XbaI

Device 1

First Round of Cloning

1.1b SoxR/S Promoter +
(Synthesized part # 54) +

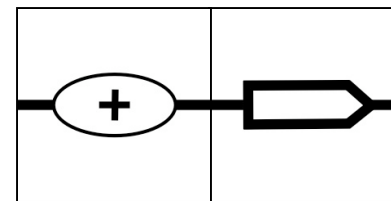
Blh parts
(Synthesized part # 57)



B0034 + k223000 + B0015 + k2230001
(RBS) (SoxR gene) (TT) (SoxS Promoter)

Cut w/ EcoR1/Spe1

+



B0034 + k223035
(RBS) (Blh gene)

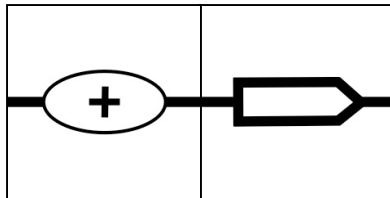
Cut w/ EcoR1/XbaI

Device 1

First Round of Cloning

1.1c Blh gene + Terminator

(Synthesized part # 57) + (BioBrick part # B0015)

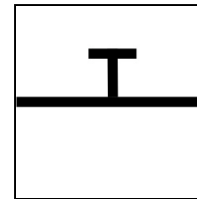


B0034 + k223035

(RBS)

(Blh gene)

+



B0015

(TT)

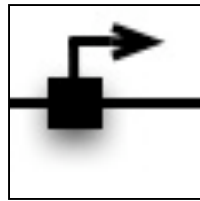
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

Device 1

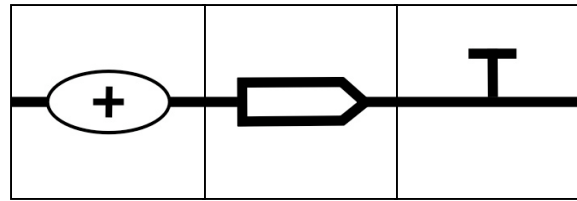
First Round of Cloning

1.1d Promoter + Blh gene w/ Terminator
(# J23100) + (Part from Cloning 1.1c)



J23100
Promoter)

+



B0034 + k223035 + B0015
(RBS) (Blh gene) + (TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

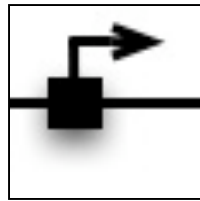
Device 1

Keep!!!

First Round of Cloning

1.1e Promoter + Crt Cluster (Beta-Carotene Producers)

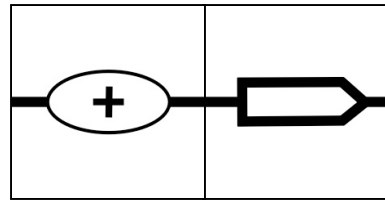
(# J23100) + (Synthesized Part on way)



J23100

Promoter)

+



B0034 + (?)

(RBS)

(crt genes = B,E, I, Y, and X)

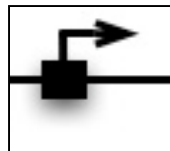
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

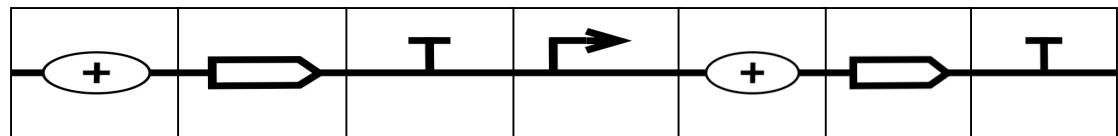
Device 1

Second Round of Cloning

1.2a Promoter + SoxR/S with GFP tag
(J23100) + (Made From cloning step 1.1a)



+



J23100

Promoter)

B0034 + k223000 + B0015 + k2230001+ B0034 +E0040 + B0015

(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (GFP) (TT)

Cut w/ Spe1/Pst1

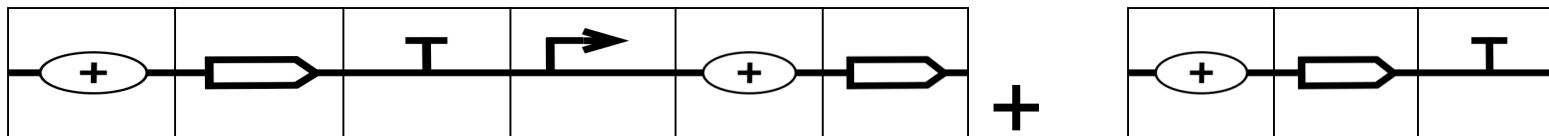
Cut w/ Xba1/Pst1

Device 1

Second Round of Cloning

1.2b SoxR/S w/ Blh + RALDH II group

(Cloned part from 1.1b) + (Synthesized part # 52)



B0034 + k223000 + B0015 + k2230001 + B0034 + k223035
(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (Blh gene)

B0034 + k223036 + B0015
(RBS) (RALDH II gene) (TT)

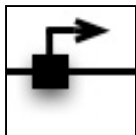
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

Device 1

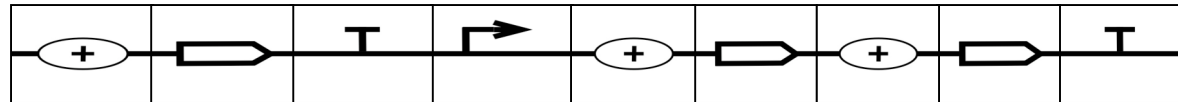
Third Round of Cloning

1.3a Promoter + SoxR/S w/ Blh and RALDH II groups
(Part #J23100) + (Cloned from step 1.2b)



J23100
Promoter

+



B0034 + k223000 + B0015 + k2230001 + B0034 + k223035 + B0034 + k223036 + B0015
(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (Blh gene) (RBS) (RALDH II gene) (TT)

Cut w/ Spe1/Pst1

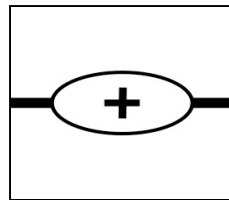
Cut w/ Xba1/Pst1

Device 2

First Round of Cloning

2.1a RBS + Il-6 and HlyAs linker

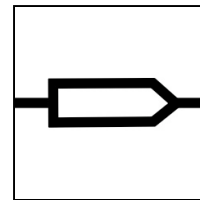
(# B0034) + (Synthesized part # 53)



B0034

(RBS)

+



K223022

(Il-6 + HlyAs linker)

Cut w/ Spe1/Pst1

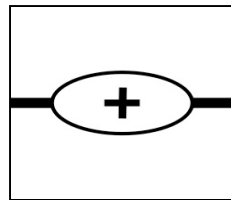
Cut w/ Xba1/Pst1

Device 2

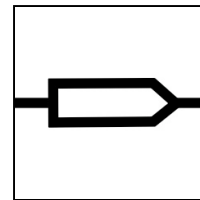
First Round of Cloning

2.1b RBS + TolC gene

(# B0034) + (Synthesized part)



+



B0034

(RBS)

K223024

(TolC gene)

Cut w/ Spe1/Pst1

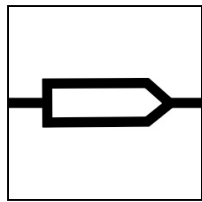
Cut w/ Xba1/Pst1

Device 2

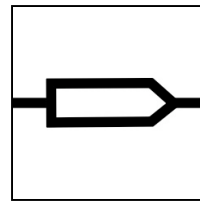
First Round of Cloning

2.1c HlyA signal + YFP

(will be registered) + (BioBricks part# I75700)



+



#

(HlyA signal0)

I75700

(YFP)

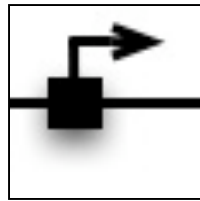
Cut w/ NgoMIV/AgeI

Cut w/ NgoMIV/AgeI

Device 2

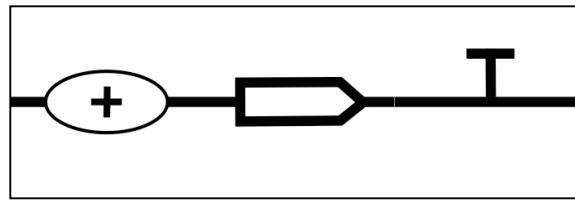
First Round of Cloning

2.1d Const. Promoter + Trp Repressor
(#J23100) + (will be registered)



J23100
(Promoter)

+



#?

(Synthesized de novo as 1 part =
RBS + trpR + TT)

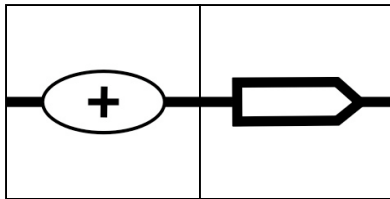
Cut w/ ?

Cut w/ ?

Device 2

Second Round of Cloning

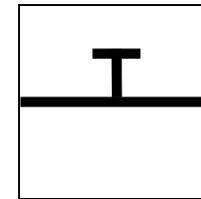
2.2a RBS w/ Il-6 and HlyA + Terminator
(Cloned from step 2.1a) + (#B0015)



B0034 + k223022

(RBS) (Il-6 and HlyAs linker)

+



B0015

(TT)

Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

Device 2

Second Round of Cloning

2.2b RBS w/ TolC

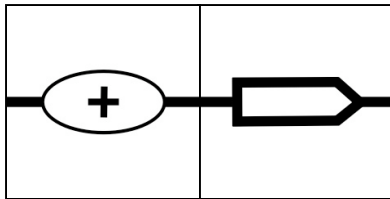
+

Hly R,B,D,C cluster

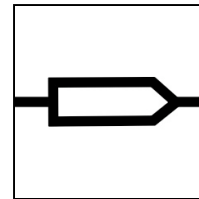
(Cloned from step 2.1c)

+

(Receiving in Plasmid from another lab)



+



B0034 + k223024

(RBS)

(TolC gene)

k223025

(Hly R,B,D,C)

Cut w/ EcoR1/Spe1

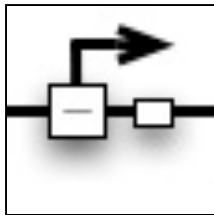
Cut w/ EcoR1/XbaI

Device 2

Third Round of Cloning

2.3a Trp Operator + Rbs w/ Il-6 and HlyAs w/ TT

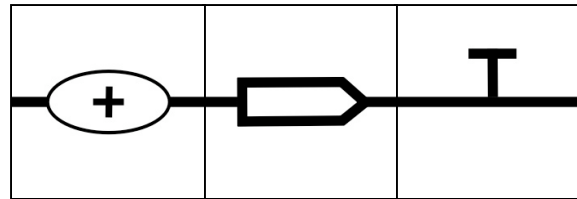
(will be registered) + (Cloned from step 2.2a)



#k223023

(Trp operator - synthesized)

+



B0034 + k223022 + B0015

(RBS) (Il-6 and HlyAs linker) (TT)

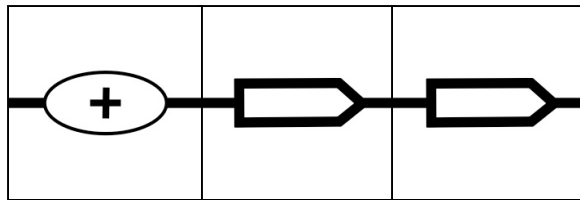
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

Device 2

Third Round of Cloning

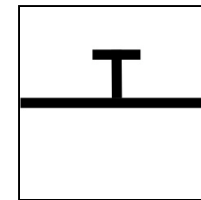
2.3b RBS w/ TolC and HlyR,B,D,C + Terminator
(Cloned from step 2.2c) + (#B0015)



B0034 + k223024 + k223025
(RBS) (TolC) (HlyR,B,D,C)

Cut w/ EcoR1/Spe1

+



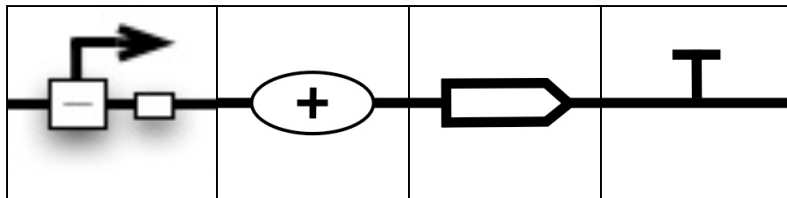
B0015
(TT)

Cut w/ EcoR1/XbaI

Device 2

Fourth Round of Cloning

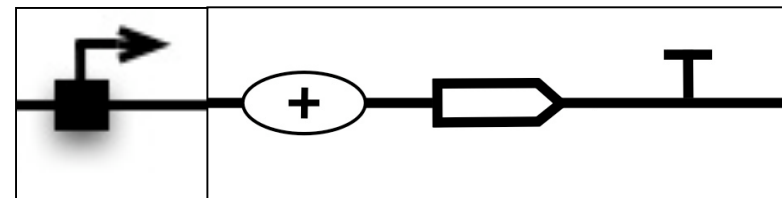
2.4a Trp Operator, RBS, Il-6+HlyA, TT + Rbs, TrpR, TT
(Cloned from step 2.3a) + (Cloned from step 2.1e)



#k223023 + B0034 + k223022 + B0015

(Trp operator-) (RBS) (Il-6 and HlyAs linker) (TT)
synthesized)

Cut w/ EcoR1/Spe1



J23100

(Const. Promoter)

+

#?

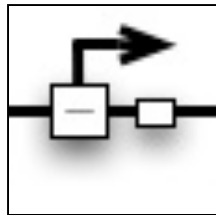
(Synthesized de novo as
1part = RBS + trpR + TT)

Cut w/ EcoR1/XbaI

Device 2

Fourth Round of Cloning

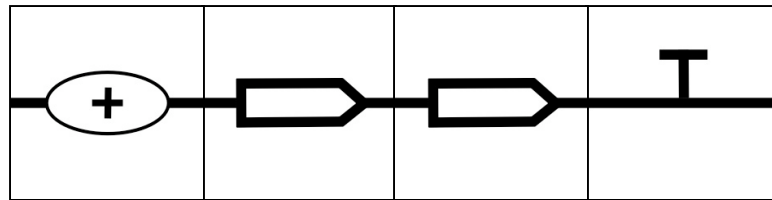
2.4b Trp Operator + Rbs, TolC, HlyR,B,D,C, TT
(will be registered) + (Cloned from step 2.3b)



#k223023

(Trp operator - synthesized)

+



B0034 + k223024 + k223025 + B0015

(RBS)

(TolC)

(HlyR,B,D,C)

(TT)

Cut w/ ?

Cut w/ ?

